OPERATING INSTRUCTIONS

LEICA MM DM4000 epi-fl microscope

You must not operate this equipment without prior training from a BALM facility staff member.

To arrange training and for help please contact:

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Standard Operating Procedure — Basic instructions

How to turn the equipment on:

1. Switch on the metal halide lamp
2. Switch on the microscope (the switch is on the back RHS of the microscope)
3. Switch on the computer and log in

How to turn the equipment off:

1. Switch off computer
2. Switch off Microscope
3. Switch off metal halide lamp

Rules of use:

This microscope should be treated with respect and care at all times.

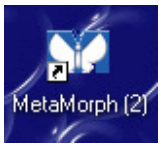
This Microscope can only be used by Masters by Research or PhD students, Postdocs and members of staff.

The microscope lenses must be cleaned after every usage and the equipment treated carefully at all times.

If you have any problems at all with the microscope, no matter how trivial they may seem please see a technician immediately.

REMEMBER: You have 5GB of disk space on this microscope. Check before you start if you have room for your experiment. If not, delete your old data.

1) Open **Metamorph** software
(basic or standard user)



NB: The software takes a while to load

2) Place slide on microscope stage and choose objective lens

Objectives available:

4x, 10x, 20x, 40x – air
40x, 63x – oil

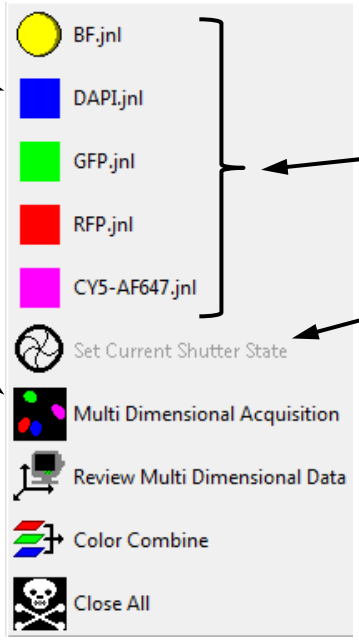
Move by hand, not automated

NB: please LOWER THE STAGE before changing between objectives (to avoid crashing lens onto slide)

3) Check rod on LHS is pushed IN to direct the light to the eyepiece

4) Select a channel from the tool bar on the RHS, and find your sample

5) Select **Multi Dimensional Acquisition**
(a new window will appear)

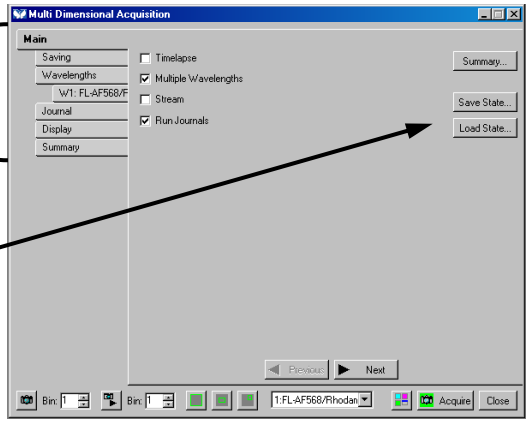


Use these buttons to change between the channels

Open and close shutter using the **shutter state** button

6) Set the parameters of your experiment using the step by step tabs in the **multi dimensional acquisition** window

7) Once you've set up your acquisition setting you can save them using the **Save State** button and reload them next session using the **Load State** button



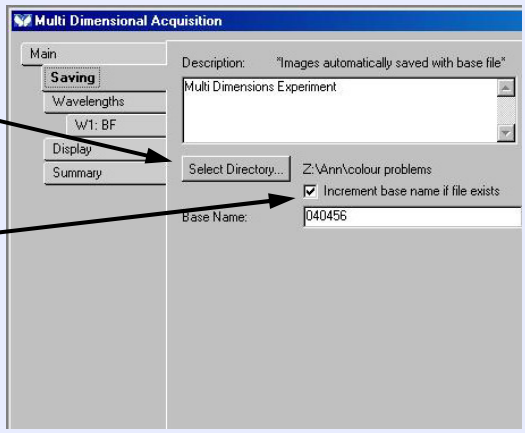
Save your data on the F-Drive

↘

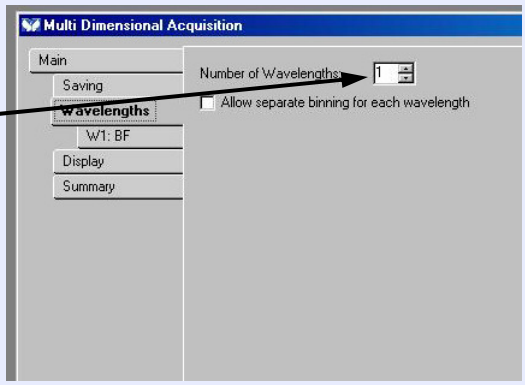
In the **Saving** tab click **Select Directory** and choose your folder on the F-Drive

Type in a **Base Name** for your experiment

Check the **Incremental base name** box is ticked (this will automatically number your images under the base name you selected)



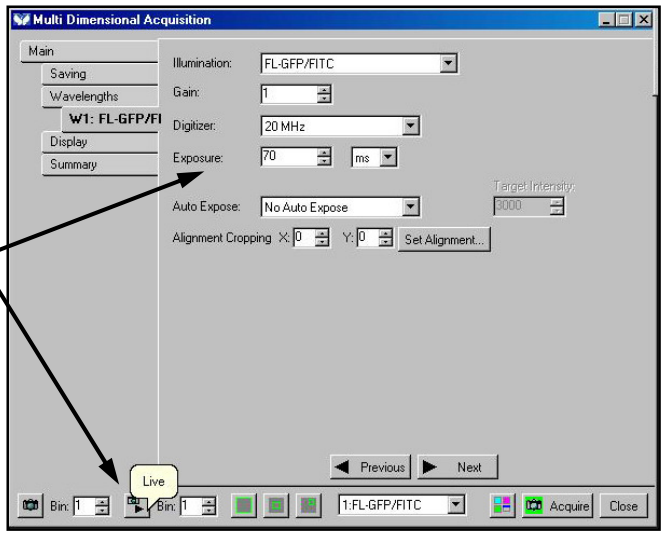
Choose the number of wavelengths you need



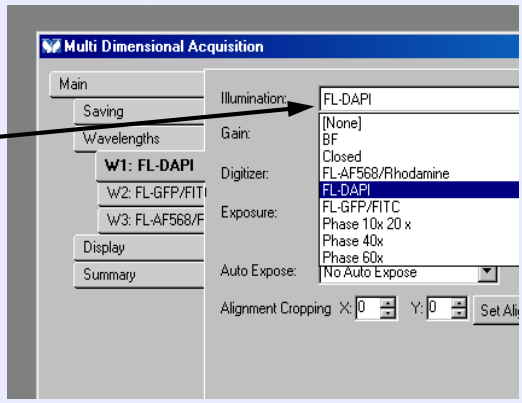
8) Pull OUT rod on LHS to direct the light to the camera

9) Click **Show Live** icon
(live image will appear on screen,
you may need to adjust the **focus**)

10) Adjust **Gain** and **Exposure** for each wavelength



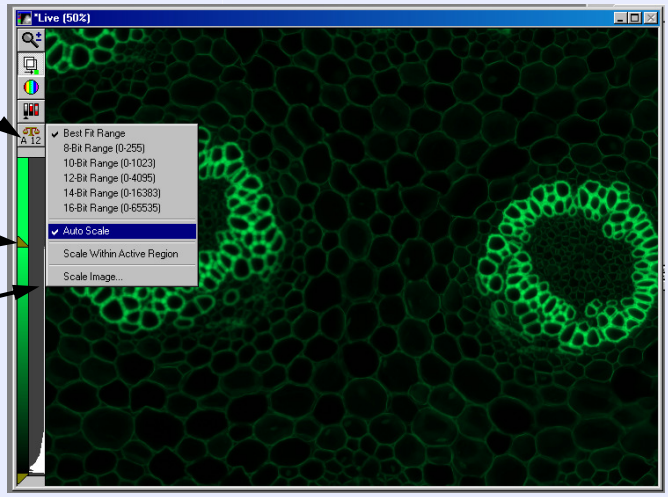
To change the assigned wavelengths select an illumination from the dropdown menu



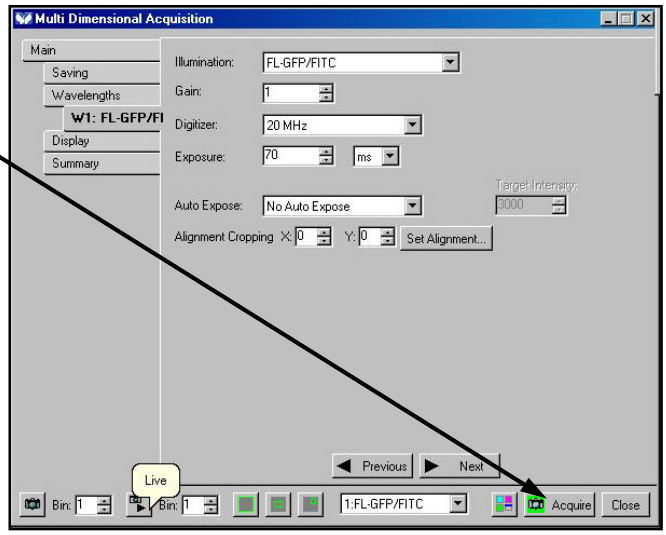
If you need to use **Auto Scale**, click on the icon on the LHS of the **Live Image Window**, and select it from the dropdown menu

Within the parameters of the gain and exposure that have been set, you can alter the image displayed by sliding the triangles on the side bar

NB. If the uppermost tail of the histogram is not viable then some areas of your image will be overexposed



11) Click **Acquire**
(a separate picture will be taken for each channel)



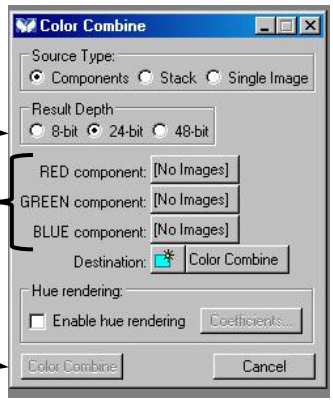
12) Creating a **Merged Image**

↘ click **Colour Combine** in toolbar on RHS

↘ select **24-bit** in **Colour Combine** window

↘ assign the appropriate images for each colour component

↘ click **Colour Combine**



↘ **Save** combined image to your folder in tiff format

When you have finished, transfer all your data to the **Z network drive**

PLEASE TIDY UP!!
Clean lenses, throw away used tissue/lens tissue, dispose of old slides in the yellow sharps bin